The Section 103 rejections are traversed. Reconsideration and withdrawal of the Section 103 rejections of the claims are requested in view of the following distinguishing comments.

The applicants do not concede that Todorov is prior art however, for completeness, the applicants provide below a substantive review of the cited art.

The Examiner will appreciate the Federal Circuit Court of Appeals has provided the following statement of the requirements for establishing a *prima facie* case of obviousness:

> Where claimed subject matter has been rejected as obvious in view of a combination of prior art references, a proper analysis under § 103 requires, inter alia, consideration of two factors: (1) whether the prior art would have suggested to those of ordinary skill in the art that they should make the claimed composition or device, or carry out the claimed process; and (2) whether the prior art would also have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success. See In re Dow Chemical Co., 837 F.2d 469, 473, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988). Both the suggestion and the reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure. Id. In re Vaeck (CA FC 1991) 20 USPQ2d 1438, 1442.

Moreover, the Examiner is urged to appreciate that

> "Obvious to try" has long been held not to constitute obviousness. In re O'Farrell , 853 F.2d 894, 903, 7 USPQ2d 1673, 1680-81 (Fed. Cir. 1988). A general incentive does not make obvious a particular result, nor does the existence of techniques by which those efforts can be carried out. In re Deuel (CA FC 1995) 34 USPQ2d 1210, 1216.

The presently claimed invention provides a method of determining the presence or absence of dysplastic or neoplastic cells in a test sample containing cells from an

individual, wherein the test sample contains a sputum specimen, bronchio-alveolar lavage specimen, urine, breast duct fluid, brushings from the alimentary tract, brushings from the cervix, a fecal cytology smear or a urine cytology smear, wherein the method involves contacting the test sample with an antibody or antibody fragment directed against Minichromosome Maintenance protein 2 (MCM2 protein); and determining the amount and/or pattern of binding of the antibody or antibody fragment to the test sample. An increase in the amount and/or a difference in the pattern, if detected for the test sample compared with normal, is indicative of the presence of dysplastic or neoplastic cells in the test sample.

The claims require the testing of cytological specimens derived from the surface of an epithelium covering or lining an organ or from a sample containing proteins and/or cells released in to a bodily fluid. Cells from certain organs may be sampled actively (for example by scraping or brushing) while many organs may be sampled passively such as, for example, by collecting cells exfoliated or sloughed spontaneously in to bodily fluids. This aspect of the presently claimed invention was described in the Amendment of March 22, 2004, for example, at pages 8-9 and is detailed in the application at, for example (with reference to the specification as presented in the parent U.S. Patent No. 6,303,323 B1), column 1, lines 48-51; column 2, lines 30-31; column 9, line 30; column 9, lines 31-36; column 9, lines 41-51; and column 13, lines 26-29.

In response to the Examiner's comments in the last paragraph of page 4 of the Office Action of June 16, 2004, the applicants submit this sampling of specific cells required by the claims, and not taught or suggested by the cited art, is the "positional

information" distinguished in the remarks of the applicants Amendment of March 22, 2004. The test samples of the claimed methods will necessarily provide information of the "expression at the surface" or "surface epithelial layers" as a patentable distinction over the cited art.

The invention of Claims 88, 89, 101, 102, 104, 105 and 107-111 would not have been obvious in view of the combined teachings of Werness et al. (Laboratory Investigation, Volume 76, No. 1, page 185A, March 1997, Abstract No. 1089) and Dunton (WO 97/16731). Consideration of the following in this regard and withdrawal of the Section 103 rejection of the claims over Werness et al. and Dunton are requested.

Werness et al. teach analysis of "Frozen, unfixed tissue from 72 tumors and 22 normal tissues" by "Western blot for BM28", which is an MCM protein of the presently claimed invention. Werness et al. also evaluated immunoreactivity in formalin-fixed, paraffin-embedded tumor and normal tissues. Werness et al. compared the presence of BM28, PCNA and Ki-67 in these samples and concluded that BM28 is

> "a novel proliferation marker which is preferentially expressed in human tumors, and which works well in formalin-fixed, paraffin-embedded tissues. Whether BM28 expression has better diagnostic or prognostic utility than other proliferation markers is currently under investigation."

Werness et al. moreover state that the percentage of BM28 positive nuclei was intermediate between PCNA and Ki-67.

Werness et al. do not teach a method involving contacting a test sample containing a sputum specimen, bronchio-alveolar lavage specimen, urine, breast duct fluid, brushings from the alimentary tract, brushings from the cervix, a fecal cytology

smear or a urine cytology smear with an antibody or antibody fragment directed against MCM2 protein.

One of ordinary skill in the art will appreciate that the test samples of the presently claimed method, which contain a sputum specimen, bronchio-alveolar lavage specimen, urine, breast duct fluid, brushings from the alimentary tract, brushings from the cervix, a fecal cytology smear or a urine cytology smear, are not histology samples, such as those tested by Werness et al., in which tissue architecture is retained. The test samples of the presently claimed invention are cytology samples which contain cells derived from the surface of the respective epithelia, sampled either actively (e.g., in brushings or smears) or passively (e.g., following exfoliation or sloughing of cells).

One of ordinary skill in the art will appreciate that test samples of the presently claimed invention are unlikely to contain expression markers from tissues below the surface or epithelial layer. Results relating to marker expression of tissue sections and expression "in human tumors", such as are described in Werness et al. would not be considered relevant to or predictive of the presently claimed invention relating to expression of MCM2 protein in the epithelial cells.

Werness et al. teach, at best, that BM28, or MCM2, is frequently present somewhere within tumor samples. The frequency of expression of BM28 in the surface layers of tissues is not taught or suggested by Werness et al. Specifically, for example, Werness et al. state that "Tumours exhibited more intense positive staining of most nuclei." The applicants believe that such a description might, for example, represent staining of the basal 51% of epithelial cells only, with no staining of the surface cells. A marker with such properties would be of no value in examination of the samples of the

presently claimed invention. See also the comments below with regard to Werness et al.'s later comment of Todorov et al.

Moreover, Werness et al. do not identify the tumors of their study. Werness et al. do state however that the "Staining intensity was similar for all three marker, but the percentage of BM28 positive nuclei was intermediate between PCNA (highest) and Ki-67 (lowest)."

The Examiner is understood to have combined Dunton as an alleged motivation for one of ordinary skill in the art to have used the BM28 of Werness et al., or the MCM2 of the present invention, in place of the Ki-67 of Dunton in samples containing cervical brushings.

The applicants submit however that Dunton states as follows with regard to the recognized use of Ki-67 in samples not containing cervical brushings (see, page 9, lines 16-22 of Dunton):

> Ki-67 is a nuclear nonhistone antigen expressed in cells that are proliferating (Gerdes J., et al. 1984 *J. Immunol.* 133:1710-1715). The recent recognition of Ki-67 as an immunohistochemical marker of dysplasia in the cervix and vulva, where it is also present in normal basal and parabasal cells, makes it a potentially interesting marker to explore in cytologic material.

According to Dunton therefore, it was only after recognition that Ki-67 was an immunohistochemical marker of dysplasia in the cervix and vulva that Ki-67 was considered a potentially interesting marker to explore in cytological material.

There is no suggestion or teaching in Werness et al. or Dunton that BM28, or MCM2, is an immunohistochemical marker of dysplasia in the cervix and vulva. See

also the comments below with regard to Werness et al.'s later comment of Todorov et al.

The Examiner has asserted "the nexus between Ki-67 and MCM2 exist[s] because Ki-67 was already a well-studied marker of cellular proliferation wherein both primary references [i.e., Werness et al., and Todorov et al.] sought it necessary to compare the immunoreaction capability of MCM2 to Ki-67." See, page 3 of the Office Action dated June 16, 2004. This alleged nexus is understood to be the basis of the Examiner's assertion that one of ordinary skill in the art would have been motivated by the cited art to make the presently claimed invention, with a reasonable expectation of success.

Dunton teaches the following on page 12, line 26 through page 13, line 6 with regard to the apparent similar reactivities of PCNA and Ki-67 in decorating the basal and parabasal nuclei of normal cervical and vulvar squamous mucosa:

> **EXAMPLES**
> **Example 1**
>
> Recent immunohistochemical studies performed in tissue sections have demonstrated that antibodies to proliferating cell nuclear antigen (PCNA) and Ki-67 decorate only the basal and parabasal nuclei of normal cervical and vulvar squamous mucosa. (Konishi I, *et al.* 1991 *Cancer* 68:1340-1350; Devictor B., *et al.* 1993 *Gynecol. Oncol.* 49:284-90; Karakitsos P., *et al.* 1994 *Gynecol. Oncol.* 55:101-107; Mittal K.R., *et al.* 1993 *Am. J. Surg. Pathol.* 17:117-122; Shurbaji M.S., *et al.* 1993 *Am. J. Clin. Pathol.* 100:22-26; Murakami T.,

> *et al.* 1993 *Acta Obst Gynaec Jpn* 45:967-72; and Raju G. 1994 *Int. J. Gynecol. Pathol.* 13:337-41)
>
> Ki-67 nuclear antigen is expressed in upper epithelial levels of intraepithelial neoplasia of the cervix and vulva, variably in condyloma, and in basal and parabasal cells of normal squamous mucosa in histologic preparations.

Dunton continues in the above however by noting that Ki-67 is also expressed in upper epithelial levels of intraepithelial neoplasia of the cervix and vulva and variably in condyloma. Dunton is silent on BM28 of Werness et al. Rather, Werness et al. comment in Todorov et al., as further discussed below, that MCM2 and Ki-67 are independent and should provide a measure of cell proliferation distinct from those offered by PCNA and Ki-67 (paragraph spanning pages 76-77 of Todorov et al.).

One of ordinary skill in the art would not have reasonably expected from Werness et al. and Dunton that BM28 would be "expressed in upper epithelial levels of intraepithelial neoplasia of the cervix and vulva and variably in condyloma" in a manner similar to Ki-67, as apparently reported by Dunton. Without knowledge of the pattern of expression of any particular marker in the surface layers of a tissue, one of ordinary skill would not have believed it reasonable to expect that the marker would be useful in the cytology applications of the claimed invention.

Furthermore, if a marker of proliferation is present in the same (or a greater) percentage of cells as Ki67 in an epithelium *overall* this does not mean or suggest that the two markers are present in the same cells, or even in the same epithelial layers. Patterns of expression of individual proteins vary according to factors such as the abundance and stability of the proteins and the differentiation state of the epithelium.

An important reason for differences in frequencies of expression is that many proteins are restricted to particular phases of the cell cycle. Ki67, for example, is not expressed in all cells in G1 phase of the cell cycle, whereas Mcm2 is. The two proteins therefore differ in their abundance and in their utility in the applications specified in the claim. To conclude, the applicants believe that without knowledge of the pattern of expression of any particular marker in the surface layers it is not possible to have any reasonable expectation that that marker would be of use in the cytology applications recited in the claims.

A key point concerning Ki67 is that if the behaviour of Mcm2 was predictable by the behaviour of Ki67, the two proteins should be expressed identically, or at least very similarly. They are not. Indeed, a key inventive step for the present claims is the observation that Mcm2 is present in substantially more cells from the surface of epithelia than is Ki67. It was not obvious to try Mcm2 staining of cytological samples based on Werness and Dunton (or Werness, Todorov and Dunton). However, even if it was obvious to try, it was certainly not obvious that Mcm2 would perform better than Ki67 in staining cells from surface layers.

The combined teachings of Werness et al., and Dunton do not provide a reasonable expectation that BM28, or MCM2, could be used as a screening marker of dysplastic or neoplastic cells of the test samples of the presently claimed methods. At best, Werness et al., and Dunton suggest that further experimentation may be performed. As noted above however, such a teaching or suggestion that it may have been "obvious-to-try" to make the claimed invention is not sufficient to establish a *prima facie* case of obviousness.

Withdrawal of the Section 103 rejection of Claims 88, 89, 101, 102, 104, 105 and 107-111 over Werness et al. (Laboratory Investigation, Volume 76, No. 1, page 185A, March 1997, Abstract No. 1089) and Dunton (WO 97/16731), is requested.

For completeness, the applicants note that the present specification demonstrates, surprisingly, dramatic differences in the potential diagnostic value of MCMs (and Cdc6) as compared with PCNA and Ki-67.

Specifically, the applicants teach the following on pages 10-11 of the specification:

> The most widely studied markers of proliferation are Ki67, a protein of unknown function and PCNA (proliferating cell nuclear antigen) (Yu and Filipe, Histochemical Journal, 1993, 25: 843-853). PCNA is involved in the elongation of DNA replication and in the mechanism of DNA repair. Therefore it is present during actual DNA synthesis by replication or repair.
>
> The present inventors have studied proteins involved in the earlier initiation stage of DNA replication. These are Cdc6 and proteins of the MCM2-7 family (MCM2, MCM3, MCM4, MCM5, MCM6 and MCM7). Williams et al (1997) (Proc. Natl. Acad. Sci. USA, 1997, 94: 142-147) reported that human HeLa Cells in culture express Cdc6 throughout proliferating cell cycles, but that WI38 human diploid fibroblasts stop expressing Cdc6 when made quiescent by serum starvation. It is shown herein that these observations extend to other cell lines and other species. MCMs are present in G1 phase nuclei (before DNA synthesis) and are progressively displaced from chromatin into the soluble nucleoplasm during DNA synthesis. It is shown herein that they too are absent from chromatin during quiescence. It is also demonstrated herein that MCM5 is absent from differentiated cells of the uterine cervix and breast.
>
> From these background facts, MCMs or Cdc6 antisera would be expected to resemble the distributions of PCNA or Ki67. Further evidence for this expectation comes from Hiraiwa et al (Int. J. Cancer, 1997, 74: 180-184) who found similar immuno-staining patterns for PCNA and MCM7 (hCDC47) in several human tissues and three types of human tumour.

The applicants tested antisera raised against human MCM protein and human Cdc6 for cervical cytology. They have studied sections of normal and diseased human uterine cervix and cervical smears and compared the results with those obtained using PCNA and Ki67. As noted above, the Examiner is understood to believe that one of ordinary skill in the art would have reasonably expected Ki-67, MCMs and, apparently, PCNA, to show similar reactivities.

The applicants have demonstrated however that Cdc6 antibodies or MCM antibodies detect LSIL (HPVI/CIN 1) lesions in the cervix more effectively than antibodies against PCNA or Ki67. Furthermore, essentially all cells of LSIL (HPVI/CIN 1) or HSIL (CIN 2/3) lesions were stained. This is in contrast to staining by other proliferation markers such as PCNA. This indicates that specific binding molecules directed to proteins of the preinitiation complex of DNA replication, particularly Cdc6 or MCM proteins (such as MCM5 but also exemplified in the specification for MCM2, MCM3, MCM4, MCM6 and MCM7) have exceptional diagnostic value for early detection of atypical or neoplastic cells. Detection with Cdc6 and MCM provides superior and unexpected results on smears, fresh and frozen samples.

The applicants have discovered therefore that target polypeptides of the present invention, such as Cdc6 and MCM proteins, may be distinguished from other cellular proliferation markers, such as Ki-67 and PCNA, which are not useful in the present invention, by being included within the preinitiation complex of DNA replication. The markers of the present invention may be distinguished by being displaced from chromatin during quiescence and differentiation. ORC2 (Gavin et al., 1995, Science 270, 1667-1671) for example, which is not a target for use in the present invention, may

be distinguished from proteins such as Cdc6 and MCM by remaining bound to chromatin in quiescent cells. ORC2 is not down-regulated in quiescent cells, though other components of the ORC complex, such as Orc1, may behave differently. Cdc6 is down-regulated rapidly during quiescence and differentiation. Cultured cells arrested in G0 for as little as 48 hours do not contain any detectable Cdc6 protein. Cdc6 is not detectable in cells arrested in vitro for longer periods of time or in differentiated cells ex vivo. Cells arrested in vitro by serum starvation or contact inhibition lose chromatin-bound MCMs (after a few days). Cells which undergo differentiation in vitro (e.g. HL-60 cells induced to differentiate with DMSO or TPA) down-regulate MCM3 but not Orc2 (Musahl, Aussois Meeting on DNA Replication, Aussois, France, June 1997). Differentiated cells from tissues ex vivo do not express MCM proteins such as MCM2 and MCM5. The six MCM proteins MCM2-MCM7 form a multiprotein complex, which splits into two subcomplexes: MCM3 and MCM5 dimer; MCM2-4-6-7 tetramer. MCM3 and MCM5 may be displaced from chromatin during S phase more slowly than MCM2-4-6-7 (Kubota et al., 1997, EMBO J. 16, 3320-3331). MCMs are chromatin-bound in G1, displaced during S phase and nuclear, but not bound to chromatin in G2. Cdc6 behaves similarly in yeast, though in addition to being displaced from chromatin it is also degraded, protein levels going down dramatically at G1/S transition. Further components of the preinitiation complex of DNA replication may be included in accordance with the present invention. Examples include human homologues of yeast components, such as Cdc7 protein kinase (Chapman and Johnston, Exp. Cell Res., 1989, 180 419-428 (yeast), Sao et al., 1997, EMBO J., 16, 4340-4351 (human--down-regulated in quiescence)), Dbf4, the regulatory subunit of Cdc7 protein kinase (Jackson

et al., 1993, Mol. Cell Biol. 13 2899-2908 (yeast), Masai et al., Cold Spring Harbor Meeting on Eukaryotic DNA Replication, Sep. 3-7, 1997 (human)), Cdcl4 protein phosphatase (Hogan and Koshland PNAS USA, 1992, 89, 3098-3102 (yeast)), Cdc45, which associates with and has a similar phenotype to MCMs (Zou et al., Mol. Cell. Biol., 1997, 17, 553-563 (yeast), Takisawa et al., Cold Spring Harbor Meeting On Eukaryotic DNA Replication, Sep. 3-7, 1997 (Xenopus)), MCM10, which associates with and has a similar phenotype to MCMs (Merchant et al., 1997, Mol. Cell Biol. 17 3261-3271). Target polypeptides of the present invention may variously be said to be any of components of the DNA pre-replicative complex, components of replication competent chromatin, involved in restricting DNA replication to once per cell cycle, components of the replication licence, involved in licensing chromatin for a single round of DNA replication, and assembled at replication origins before initiation of DNA replication.

Specifically, the specification exemplifies the relationships of Cdc6 and MCM (such as MCM5) to cell proliferation in Example 2.

It has previously been shown by Western blot that the immortal human cell line HeLa expresses Cdc6 and MCM5 throughout the cell cycle (Williams et al., Proc. Natl. Acad. Sci. USA, 1997, 94: 142-147; Schulte et al., Eur. J. Biochem., 1996, 235, 144-15 151). The down-regulation of Cdc6 in quiescent Wi38 human fibroblasts was reported by Williams et al, 1997.

The applicants have also shown by Western blot that Cdc6 expression is down-regulated when mouse 3T3 fibroblasts are made quiescent by contact inhibition. Specifically, the NIH 3T3 cell line was arrested by growing to confluence. Cultures were held in quiescence for 7 days. Cells were released from G0 arrest by trypsin

detachment and replating. Soluble (supernatant) and nuclear protein (pellet) extracts were prepared at three-hourly intervals and both extracts were immunoblotted with antibodies against human MCM5, Orc2 and Cdc6.

Orc2 (see Gavin et al. for cloning--Science (1995) 270 1667-1671) remains chromatin bound in quiescent cells (G0) and does not increase appreciably as cells enter the cell cycle. In contrast, MCM5 could not be detected in the chromatin bound fraction (pellet) of quiescent cells even though the soluble fraction contained significant amounts of MCM5. In contrast to MCM5, Cdc6 was completely absent from quiescent cells but the expression of this protein was rapidly induced as cells re-entered the cell cycle. Similar results were also obtained with the human EJ13 cell line (derived from a bladder carcinoma).

These results provide indication that the absence of Cdc6 in quiescent cells is a general phenomenon.

These studies were extended by immunofluorescence and application of anti-Cdc6 and anti-MCM5 antibodies to whole cells.

Expression of Cdc6 and of MCM5 was found to be also down-regulated when newborn human fibroblasts (NHF) are made quiescent by contact inhibition. NHF were grown to confluence and held in quiescence for three days. Cells were released from G0 arrest by trypsin detachment and replating. Whole cells were then harvested at multiple time points after release up until entry into S-phase. Staining with propidium iodide was used to reveal DNA and results were compared with staining of the samples with anti-Cdc6 and anti-MCM5 antibodies.

Quiescent (G0) cells showed no Cdc6 immunoreactivity, and a very weak signal with anti-MCM5 antibody. However, during entry into cell cycle and S-phase, strong nuclear immunoreactivity for Cdc6 and MCM5 was observed.

These studies might suggest that anti-Cdc6 antibodies could provide a marker for proliferating cells similar to PCNA or Ki67. Neither PCNA nor Ki67 has proved satisfactory however for cervical cytology.

In Example 3 of the application, anti-Cdc6 and anti-MCM5 binding molecules were demonstrated to detect abnormal (e.g., tumor) cells much more effectively than PCNA or Ki67 antibodies.

Specifically, results outlined in Example 2 might suggest that Cdc6 expression might resemble that of PCNA or Ki67, neither of which has proved satisfactory for cervical cytology. The applicants compared antibodies to these proteins on sections of normal or diseased uterine cervix.

Immunostaining of cervical squamous intra-epithelial lesions (SILs) for the conventional proliferation markers PCNA and Ki67 shows different pattern of immunoreactivity when compared with staining for Cdc6 or MCM (MCM5 in the Example of the application). In normal cervix, antibodies against all four antigens showed positive immunostaining of epithelial cells confined to the basal and parabasal layers, as might have been suggested for BM28, Ki-67 and PCNA by the combined teachings of Werness et al. with Dunton.

No immunostaining of metaplastic, stromal or inflammatory cells was observed. In both low and high grade SILs (LSIL and HSIL) antibodies against Cdc6 and MCM (i.e., MCM5 in the Example of the specification) showed positive immunostaining of the

majority (>95%) of the abnormal cells. In contrast, immunostaining for PCNA and Ki67 was positive in only a minority population (<30%) of abnormal cells in both grades of SILs. Koilocytes, which are characteristic of LSIL and reflect an HPV cytopathic effect, all showed positive immunostaining with Cdc6 and MCM (MCM5 of the Example of the specification), whereas only a minority population (20%) showed positive staining for PCNA or Ki67.

The much greater level of staining of abnormal cells according to the present invention provides a distinct advantage for using anti-Cdc6 or anti-MCM (such as MCM5) binding molecules over anti-PCNA and anti-Ki67 molecules in cervical screening wherein tissue samples are most readily taken by way of smears from the surface of the epithelium.

As cervical smears sample only a top surface layer of cells from the cervix, it is important to have a high level of staining. Such high-level staining obtained with anti-Cdc6 and with anti-MCM (MCM5 of the Example of the specification) on the early stage abnormality (low grade SIL, CIN1), not shown with anti-PCNA nor anti-Ki67, is very significant. Furthermore, the full thickness staining obtained using anti-Cdc6 and anti-MCM (MCM5 of the Example of the specification) antibodies on LSIL samples, but not anti-PCNA nor anti-Ki67 antibodies, highlights the particular usefulness of the former for assessing smear samples for early stage potential pre-malignancy.

These advantages and differences would not have been reasonably expected from the teachings of Werness et al., and Dunton.

Example 4 of the application demonstrates the use of Cdc6 and MCM5 antibodies to detect abnormal cells in cervical smears. As noted above, Example 3 of

the application demonstrates the value of anti-Cdc6 and anti-MCM (MCM5 in the Example of the specification) binding molecules for detecting potentially pre-malignant lesions in sections of the uterine cervix. Further experimental results show that they are equally effective in detecting abnormal cells in cervical smear preparations.

Examples of normal cervical smears showed a characteristic strip of parallel arranged endocervical cells and a mixed population of superficial and metaplastic squamous cells. There was no evidence of specific anti-Cdc6 or anti-MCM5 immunoreactivity with any of the antibodies tested.

Abnormal smears containing dyskaryotic cells (atypical squamous cells) showed positive staining with three different anti-Cdc6 antibodies and with an anti-MCM5 antibody. Koilocytes also showed strong immunoreactivity with anti-Cdc6 and anti-MCM5 antibodies. None of the adjacent normal superficial squamous/metaplastic cells shows Cdc6 or MCM5 immunoreactivity.

Results were obtained using different anti-Cdc6 antibodies, preferentially staining LSIL cells, including koilocytes, against a very low background in smears of normal cervix, or in normal cells in smears from abnormal cervix. Similar results were seen with antibodies against MCM5.

The exemplifications of the application, as for example summarized above, demonstrate the unexpected benefits of the present invention, as compared with the detection of Ki-67 and/or PCNA of the cited art.

Withdrawal of the Section 103 rejection of Claims 88, 89, 101, 102, 104, 105 and 107-111 over Werness et al. (Laboratory Investigation, Volume 76, No. 1, page 185A, March 1997, Abstract No. 1089) and Dunton (WO 97/16731), is requested.

The invention of Claims 88, 89, 101, 102, 104, 105 and 107-111 would not have been obvious in view of the combined teachings of Todorov et al. (Laboratory Investigation, January 1998, Vol. 78, No. 1, pages 73-78) and Dunton (WO 97/16731). Consideration of the following in this regard and withdrawal of the Section 103 rejection of the claims over Todorov et al. and Dunton are requested.

Todorov et al. appears to be an expanded report of the Werness et al. abstract discussed above. The authorship of Todorov et al. is the same as the authorship of Werness et al. Many of the remarks above as to the patentability of the claimed invention over Werness et al. and Dunton are applicable in response to the Examiner's rejection of the claims over the combination of Todorov et al. and Dunton. The above comments will not be repeated however the Examiner is again requested to review the above comments as they relate to Dunton and the combination of Werness et al. therewith.

Todorov et al. identifies BM 28 as MCM2 (or "HsMCM2" for *Homo sapiens* MCM2). In the Discussion, Todorov et al. state that expression levels of MCM2 in normal tissues was detected most readily in "those that possess a significant population of proliferating cells, such as colon epithelium." See, page 76, first full paragraph of Todorov et al. Similar to Ki-67 noted above in the quoted passage from Dunton's teaching regarding Ki-67, Todorov et al. teaches the MCM2 was detected in normal tissues in "only the proliferative components - the basal layer of the epidermis, the crypts of both the small and large intestines, and the germinal centers of lymphoid tissue." See, page 74, right column, last paragraph (emphasis added). Todorov et al. continues that "a typical colon carcinoma sample ... shows a marked increase in both

the number of positive nuclei and in the intensity of the nuclear staining." See, sentence spanning pages 74-75 of Todorov et al. Todorov et al. does not indicate state, teach or suggest that MCM2 was expressed in "upper epithelial levels" in intraepithelial neoplasia or invasive neoplasia, as well as in the normal basal layer, as was indicated as being important for Dunton in relying on Ki-67 as a marker in cervical samples.

There is no suggestion in Todorov et al. therefore that MCM2 expression in epithelial cell brushings and/or liberated in to bodily fluids could be useful in determining the presence or absence of dysplastic or neoplastic cells, as presently claimed.

Dunton is not believed to cure the deficiencies of Todorov et al. Moreover, Todorov et al. is believed to teach away from the combination of Todorov et al with Dunton. Specifically, for example, Todorov et al. teaches that

> "HsMCM2 is associated with cell proliferation independently of PCNA and Ki-67. Therefore, it should provide a measure of cell proliferation distinct from those offered by PCNA and Ki-67. This possibility is supported by evidence of the different levels and intensities of staining produced by antibodies against these three proteins." See, paragraph spanning pages 76-77 of Todorov et al. (emphasis added).

Accordingly, as Todorov et al. teach that MCM2 expression is independent and distinct from expression of Ki-67, the Examiner's conclusion that one of ordinary skill in the art would allegedly have measured MCM2 in place of Ki-67 in the method of Dunton would have been contrary to the teaching of Todorov et al.

Withdrawal of the Section 103 rejection of claims 88, 89, 101, 102, 104, 105 and 107-111 over the combined teachings of Todorov et al. (Laboratory Investigation, January 1998, Vol. 78, No. 1, pages 73-78) and Dunton (WO 97/16731) is requested.

The claims are submitted to be in condition for allowance and a Notice to that effect is requested.

Respectfully submitted,

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